MONOCHROMATIC X RAY RADIOGRAPHIC ANALYSIS OF CALCIUM IN WHEAT

by

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INTRODUCTION

The elemental constituents of many biological specimens are well known but their exact locations within the specimen are not known. In individual wheat kernels for example, it was not known whether the endosperm was such that its mineral elements were homogeneously or heterogeneously distributed. Trying to supply an answer to this question Cereal Chemists (3,4) probed different regions of wheat endosperm with small drills, and then analyzed the ash obtained from the drillings. They concluded that the endosperm was heterogeneous, but were unable to exactly pinpoint mineral segregations. This situation lead to the present use of fluorescent x rays to map and measure the magnitude of calcium segregations within thin sections of wheat endosperm by radiography. To explain why this is possible, how it is done, and the results of the calcium analysis is the purpose of this thesis.

When a chemical element is irriadiated with a carefully chosen polychromatic x ray beam monochromatic x rays characteristic of this element are produced; secondary characteristic x rays so produced are known as fluorescent x rays. These fluorescent x rays can then be used to obtain radiographs of thin specimens. This technique is known as fluorescent x ray radiography.

The genesis of fluorescent x ray radiography was the need for monochromatic x ray beams which are necessary for radiographic chemical analysis. The physical properties of many elements

forbid their use as x ray tube targets but allows them to be used as secondary fluorescent x ray targets external to the x ray tube. Thus by using fluorescence techniques the experimentalist has a greater number of monochromatic x ray beams at his disposal. Realizing this Engstrom (1) constructed a special tube for fluorescent radiography and successfully completed an x ray chemical analysis of thin sections of muscle tissue.

Fluorescent radiography received a great impetus with the development of a thin beryllium window x ray tube (2,5), a source of high intensity, long wavelength x rays, for it was shown that this tube could be used to obtain highly monochromatic fluorescent x ray beams (6). A check of the degree of monochromaticity (6) of fluorescent x ray beams obtained with a Machlett AEG-50 x ray tube was made by absorption. This check, however, did not show the precise x ray tube voltages at which the fluorescent beam was the highest degree of monochromaticity, but a second investigator (7) showed that highest monochromaticity resulted when the potential across the x ray tube was 2-3 times the energy of the fluorescent line to be excited in the secondary target. It is necessary to operate the x ray tube with this result in mind for scattered radiation also blackens the x ray film and introduces considerable error into the analysis.

Use of the beryllium window tube and a specially constructed camera for fluorescent microradiography of metal alloy foils has

been reported elsewhere (9).

Although fluorescent x ray radiographic analysis has been limited in application, it is potentially a prime technique for probing biological materials.

THEORY

The Production of X Rays

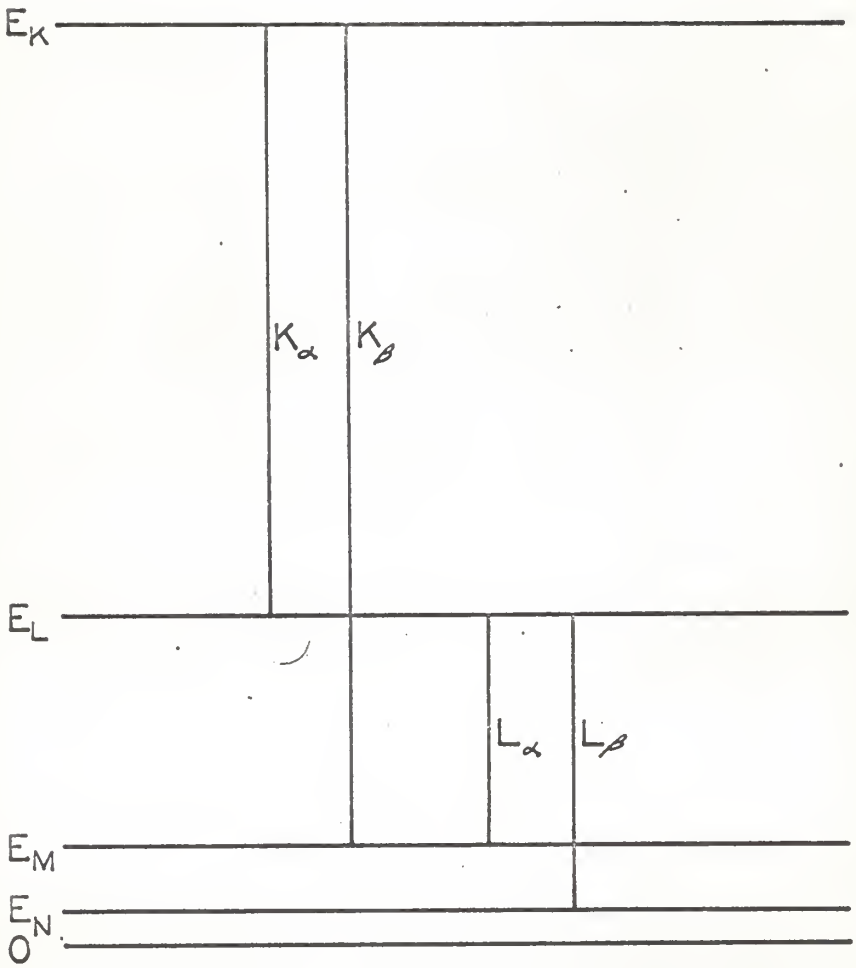
X rays are produced when matter is bombarded with energetic electrons. When energetic electrons strike a target material two different x ray spectra are produced; one is a continuous spectrum, originating from energy radiated by the incident electrons when they are stopped by the target material, and the other is a discrete line spectrum characteristic of the target atomic constituents. The origin of the discrete line spectrum is complicated: The incident energetic electron beam removes inner shell electrons from some of the target atoms, leaving these atoms ionized. The inner shell vacancies are then filled by electrons from neighboring outer shells, and as result of these electronic transitions, discrete, characteristic x rays are emitted from the target atoms. The x ray spectrum from an ordinary x ray tube consists of a discrete line spectrum, characteristic of the tube target material, superimposed on a continuous spectrum.

X rays characteristic of a material are also produced when the material is irradiated with sufficiently energetic x rays.

EXPLANATION OF PLATE I

This is a simplified x ray energy level diagram. The energy increases vertically, e. g. $E_M < E_L < E_K$. K_α radiation is emitted when an orbital electron goes from the L shell to the K shell, and the energy of the atom goes from E_K to E_L.

PLATE I



A second target material is placed in front of an x ray tube window and irradiated with the high intensity primary x ray beam from the x ray tube. The primary x rays photoelectrically remove inner shell electrons from the secondary target atoms leaving these atoms ionized. Characteristic fluorescent x rays are then emitted from the secondary target atoms.

Fluorescent x ray production may be visualized by use of the simplified x ray energy level diagram appearing in Plate I. A K-shell electron is bound to an atom with energy E_K, and an incident x ray photon possessing energy greater than or equal E_K can photoelectrically remove this electron from the atom. When this electron has been removed from the atom then the atom is said to be in an excited energy state E_K. An electron from a neighboring outer shell can then fill the vacancy in the K-shell. Supposing this vacancy to be filled by an L-shell electron the atom goes to the excited energy state E_L, and due to the electron transition the atom surrenders energy $E_K - E_L$ in the form of a fluorescent x ray of wavelength λ_{K_α},

$$\lambda_{K_\alpha} = \frac{hc}{E_K - E_L} \quad ;$$

where h is Planck's constant and c is the speed of light in vacuum. Electronic transitions from the L-shell to the K-shell produces K_α radiation, and transitions from the M-shell to the K-shell produces K_β radiation, where

$$\lambda_{K_\beta} = \frac{hc}{E_K - E_M}$$

A similar argument for photoelectric removal of L-shell electrons and resulting L_α and L_β radiation is also valid. The subscripts α and β respectively denote the most intense and the second most intense emission lines.

The Production of Monochromatic X Ray Beams

A nearly monochromatic x ray beam can be obtained from an ordinary x ray tube by filtering. Filtering consists of placing a material of atomic number 1 or 2 less than the atomic number of the x ray tube target in front of the x ray tube window. Upon penetrating this filtering material the intensity of the continuous spectrum from the x ray tube is greatly reduced in proportion to the intensity of the characteristic radiation from the tube target. Thus this leaves a sufficient amount of the most intense characteristic line of the target material plus a small residue of the continuous spectrum. For many applications the filtered beam can be considered to be monochromatic, but it leaves something to be desired when used for the identification of mineral segregations in biological specimens.

The thickness of the filter is in general chosen such that the intensity of the characteristic K_α line is reduced to one-half its initial value.

Monochromatic x ray beams are also obtained by fluorescence techniques. An external secondary target material is placed in front of an x ray tube window and irradiated with high intensity primary x rays; this induces x ray fluorescence of the secondary target. When the potential across the x ray tube is 2 or 3 times the excitation energy of the characteristic x ray to be excited in the secondary target material, a highly monochromatic fluorescent x ray beam results. The fluorescent radiation emitted perpendicular to the primary x ray beam is used since at this angle the scattering of the primary radiation is a minimum. The fluorescence yield of the secondary target is small and is dependent upon the wavelength of the primary radiation. An x ray tube with a thin beryllium window must be used as the primary source if the intensity of the fluorescent beams, in the 1-10 Angstrom range, is to be adequate for radiography of biological specimens. The fluorescent x ray attachment which was specially constructed for monochromatic fluorescent x ray radiography of thin sections of wheat is described in the apparatus section.

Bragg reflection of polychromatic x ray beams by crystal planes is also used to obtain monchromatic x ray beams of low intensity. This technique, however, is beyond the scope of this presentation and will not be discussed further.

The Absorption of X Rays

The decrease in the intensity of a collimated monochromatic x ray beam when passed through a monoelemental absorber is described by the equation $I = I_o \exp(-\mu m)$, where I_o and I are respectively the initial and final beam intensity, μ is the mass absorption coefficient of the element, and m is the mass per unit area of the absorber expressed in gm/cm^2. This intensity decrease is due to scattering, and to photoelectric absorption of the incident x ray beam by inner shell electrons of the absorber atoms.

The mass absorption coefficient, μ, increases with increasing wavelength, λ, approximately as $k \lambda^3$, k being a proportionality constant, until a critical wavelength, λ_K; characteristic of the K shell of the absorber element is reached. A definite decrease in the mass absorption coefficient occurs at λ_K; this is attributable to a decrease in photoelectric interactions between the incident x ray beam and electrons of the absorber atoms. X rays of wavelength greater than λ_K are energetically insufficient to remove $1S_{\frac{1}{2}}$ electrons from the absorber atoms, thus at these wavelengths less of the incident beam is absorbed by the element, and the sudden decrease in the mass absorption coefficient is observed.

From λ_K the mass absorption coefficient increases with increasing wavelength approximately as $k' \lambda^3$, k' being a different

proportionality constant, until a second critical wavelength, λ_{Li}, characteristic of the L_i sub-shell of the absorber element is reached. A definite decrease in the mass absorption coefficient occurs at λ_{Li}; this is again attributable to a decrease in photoelectric interactions between the incident x ray beam and electrons of the absorber atoms. X rays of wavelength greater than λ_{L_i} are energetically insufficient to remove $2S_{\frac{1}{2}}$ electrons from the absorber atoms, thus again less of the incident beam is absorbed by the element, and a second sudden decrease in the mass absorption coefficient is observed. Other decreases in the mass absorption coefficient occur at wavelengths λ_{Lii} and λ_{Liii}, where the incident beam becomes energetically insufficient to remove $2P_{\frac{1}{2}}$ and $2P_{\frac{3}{2}}$ electrons respectively.

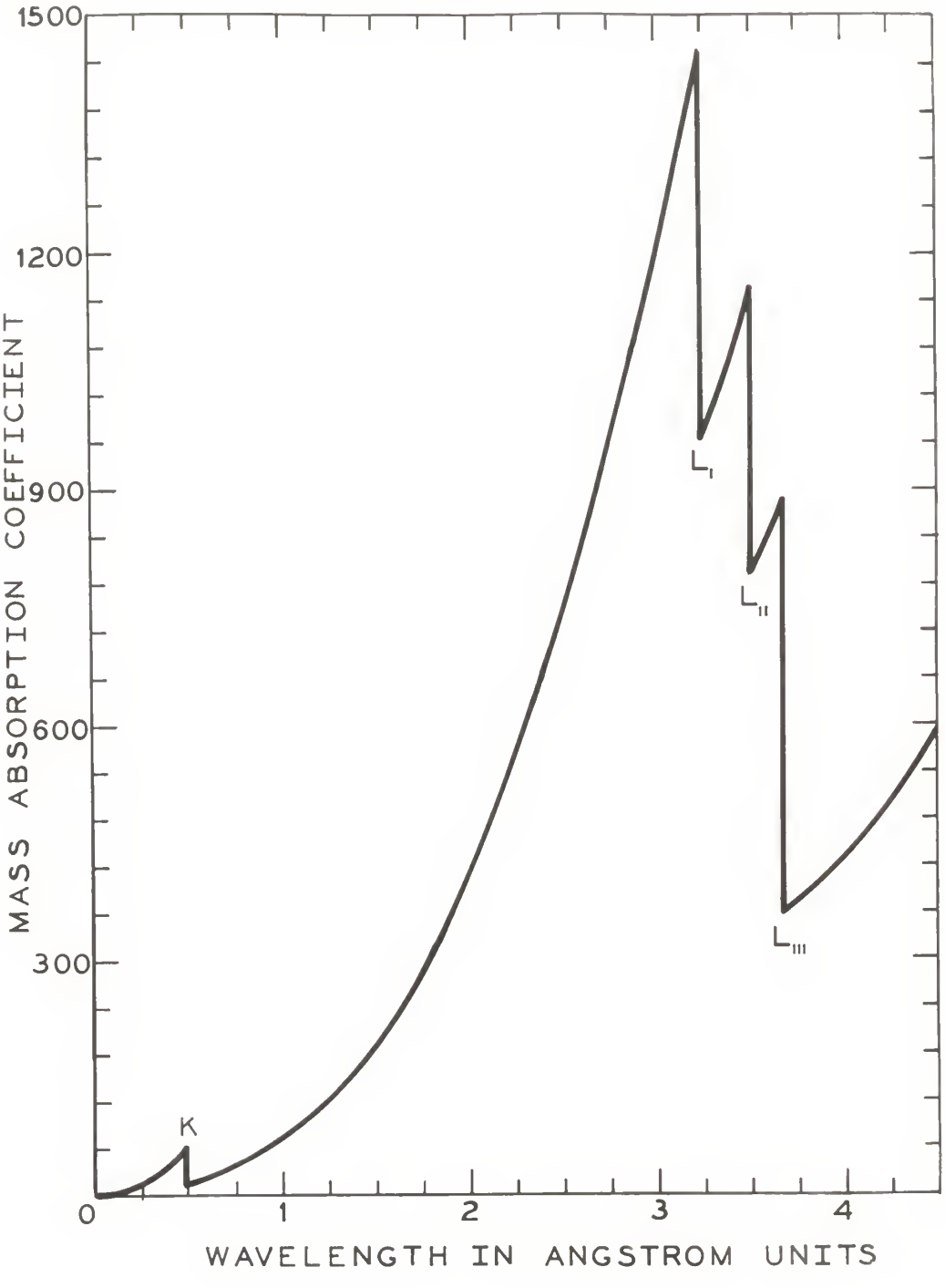
The definite decreases in the mass absorption coefficient at the critical wavelengths are known as critical absorption edges; they occur at wavelengths which are characteristic of the absorber element. Plate II shows a plot of μ vs λ for silver.

Wheat is polyelemental thus it is necessary to discuss the x ray absorption properties of such a material. When a group of elements are chemically combined there is a small shift in the characteristic x ray absorption edges of the individual elements. These shifts are so small that they may be neglected and it follows that the characteristic x ray absorption properties of a particular element are independent of the elements chemical state.

EXPLANATION OF PLATE II

This is a plot of the mass absorption coefficient verses x ray wavelength for silver. The K, L_i, L_{ii}, and L_{iii} absorption edges occur at wavelengths 0.4858, 3.23824, 3.4947, and 3.6728 Angstroms respectively.

PLATE II


1500
1200
900
600
300
0
MASS ABSORPTION COEFFICIENT
K
L_I
L_{II}
L_{III}
1
2
3
4
WAVELENGTH IN ANGSTROM UNITS

This is true for the chemical combination of atoms is dependent upon outer shell valence electrons while characteristic x ray absorption is dependent upon inner shell electrons.

In mathematical form the decrease in the intensity of a collimated monochromatic x ray beam when passed through a polyelemental absorber is also described by the equation $I = I_o \exp(-\mu m)$.

There is, however, in this case, an implicit difference in μ the mass absorption coefficient. Supposing the polyelemental absorber to be composed of k elements, its mass absorption coefficient, μ_i, at a wavelength λ_i, is given by

$$\mu_i = \sum_{j=1}^{k} a_j \mu_{ji} \quad ,$$

where a_j is the fractional mass of the specimen which is composed of the elements of the j^{th} kind, and μ_{ji} is the mass absorption coefficient of the j^{th} element at wavelength λ_i.

RADIOGRAPHIC MAPPING OF CALCIUM SEGREGATIONS

The wavelength at which the K critical absorption edge of a particular element is observed is independent of the physical and chemical state of that element. This is precisely the physical phenomenon we exploit to detect calcium segregations in wheat. Wheat consists primarily of carbon, nitrogen, and oxygen (CNO), and in general for the 1-10 Angstrom wavelength range CNO has a low x ray mass absorption coefficient relative to that of calcium.

Curve B in Plate III is a log-log plot of the mass absorption coefficient against x ray wavelength for plant protein. A similar curve can be plotted for carbohydrate material.

To map calcium segregations it is noted from Plate III that the K absorption edge of calcium occurs at 3.070 Å, and that this absorption edge is bracketed by λ_{Ti}, the titanium characteristic K fluorescent wavelength at 2.750 Å, and by λ_{Ca}, the calcium characteristic K fluorescent wavelength at 3.360 Å. A radiograph of the specimen is then taken with each of the fluorescent monochromatic x ray beams of wavelength λ_{Ti} and λ_{Ca}. It is shown in Plate III that the beam of wavelength λ_{Ti} will be absorbed more than the beam of wavelength λ_{Ca} at points where calcium concentrations are located; the two radiographs record this as visible contrast differences. The visual contrast differences between the two radiographs map the positions of the calcium segregations.

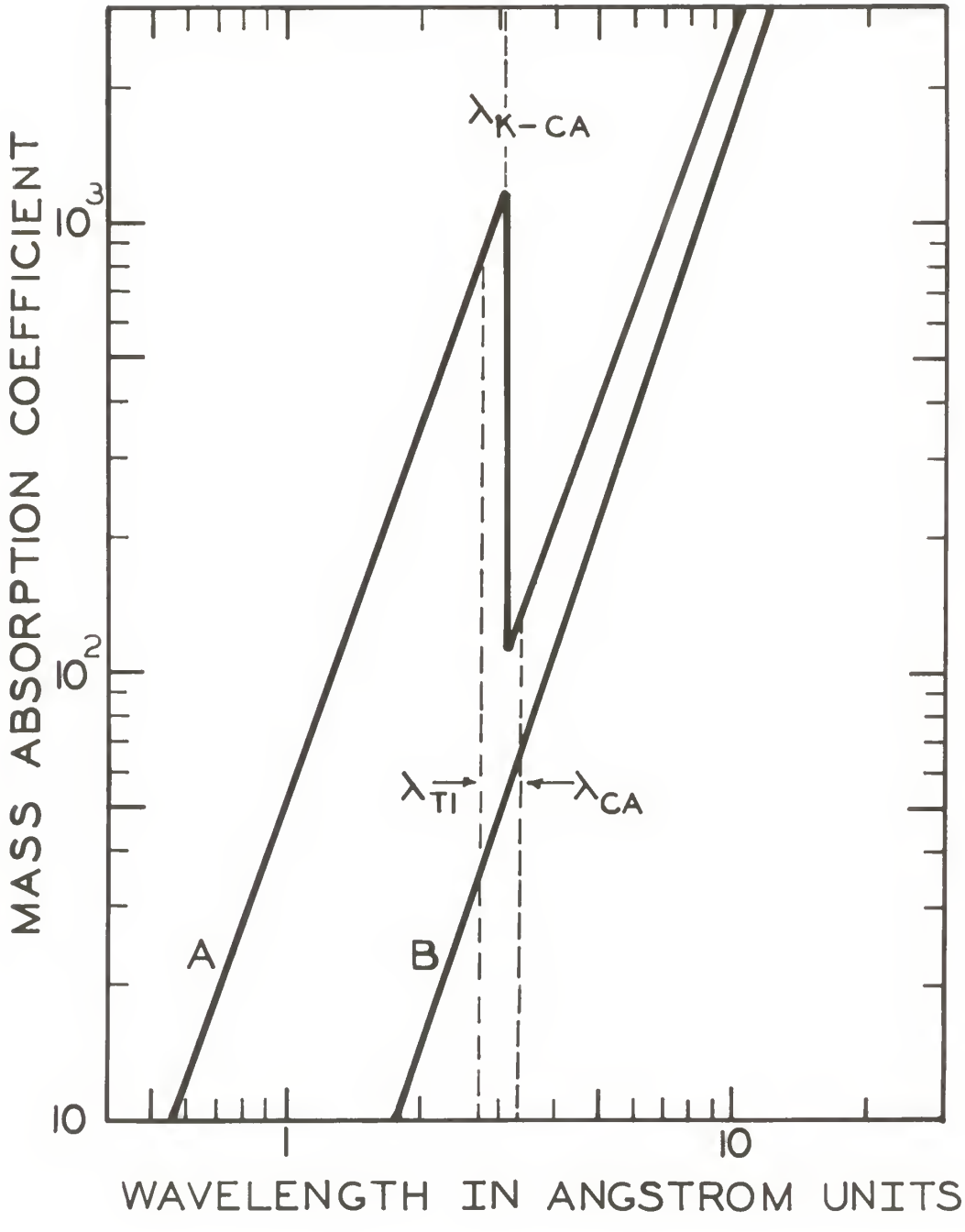
An example of this as applied to a logitudinal section of Wichita hard red winter wheat may be seen in Plate IV, Figures 1(A) and 1(B) which are positive enlargements made from two radiographs taken with fluorescent x ray beams of wavelengths λ_{Ti} and λ_{Ca}. Since these are positive enlargements the white areas correspond to regions in which the specimen absorbed less of the incident fluorescent x ray beam. Note that in the print, Figure 1(A), of the radiograph taken with wavelength λ_{Ti}, for which the x ray absorption coefficient of calcium is high, there are fewer

EXPLANATION OF PLATE III

Curve A is a log-log plot of mass absorption coefficient versus wavelength for the element calcium. Curve B shows the mass absorption coefficient of plant protein, assuming it to consist of 6%H, 54%C, 17%N, and 23%O.

$\lambda_{Ti} = 2.75\text{Å}$, $\lambda_{K\text{-}Ca} = 3.07\text{Å}$, $\lambda_{Ca} = 3.36\text{Å}$.

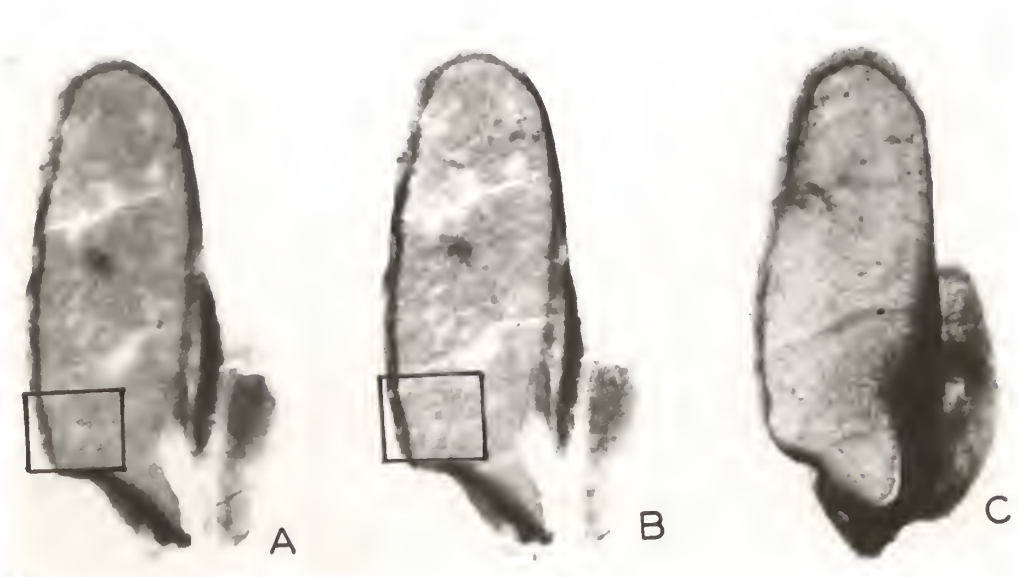
PIATE III


MASS ABSORPTION COEFFICIENT
10^3
10^2
10
λ_{K-CA}
λ_{TI}
λ_{CA}
A
B
1
10
WAVELENGTH IN ANGSTROM UNITS

EXPLANATION OF PLATE IV

Shown are positive enlargements of radiographs obtained with fluorescent radiation (A) of wavelength λ_{Ti} at 15 PKV, 20 ma x 30 min, (B) of wavelength λ_{Ca} at 12.5 PKV, 25 ma x 100 min, and (C) a transmitted light photomicrograph. The specimen is a logitudinal section of Wichita hard red winter wheat of thickness 450 μ.

PLATE IV



distinct white areas, while in the enlargement, Figure 1(B), of the radiograph taken with wavelength λ_{Ca}, for which the x ray absorption coefficient of calcium is low, there are vivid white regions which correspond to heavy penetration of the fluorescent x ray beam. Thus in this wheat specimen there are regions in which λ_{Ti} radiation is heavily absorbed, but in which λ_{Ca} radiation is lightly absorbed; these regions are areas of high calcium concentration.

From these two enlargements calcium appears to be concentrated in the cell walls of the endosperm. For example consider the boxed areas in Figures 1(A) and 1(B). This belief is verified by the photomicrograph shown in Figure 1(C), which was obtained with transmitted light. It is seen that cell walls in Figure 1(C) correspond to areas of high calcium concentration in Figures 1(A) and 1(B).

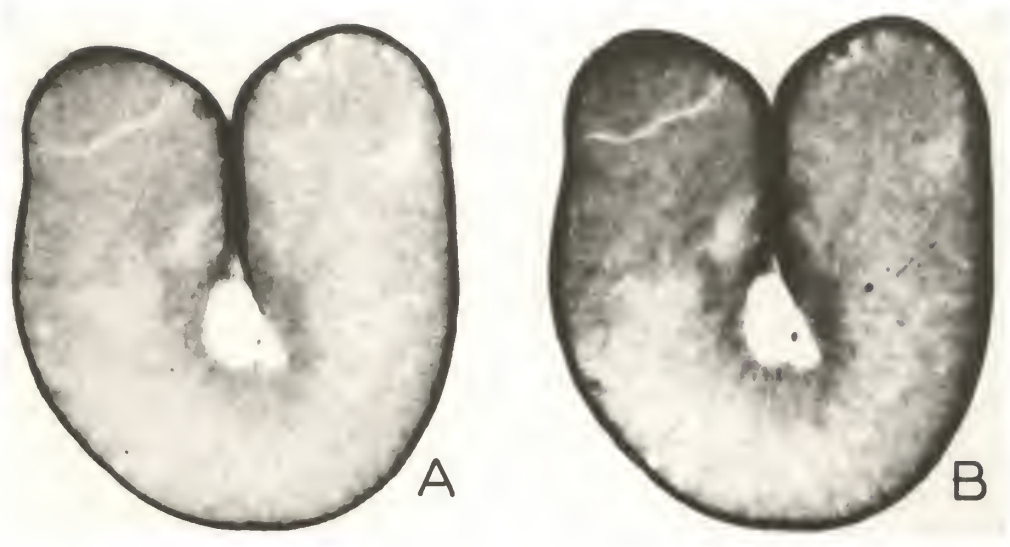
Additional enlargements of pairs of such radiographs are shown in Plates V and VI, Figures 1(A) and 1(B). The Figures in Plate V show a heavy calcium concentration within the outer and center regions (see Plate IX) of the lower part of a section of Omar white wheat. The Figures in Plate VI show a slight calcium concentration within the cell walls of a section of Selkirk hard red spring wheat, and an outstanding calcium concentration in the center, cheek, and outer regions at the upper right.

Note that in Plate V no cell wall structure can be seen. The

EXPLANATION OF PLATE V

Shown are positive enlargements of radiographs obtained with fluorescent radiation (A) of wavelength λ_{Ti} at 15 PKV, 20 ma x 30 min, and (B) of wavelength λ_{Ca} at 12.5 PKV, 25 ma x 100 min. The specimen is a transverse section of Omar white wheat of thickness 315 μ.

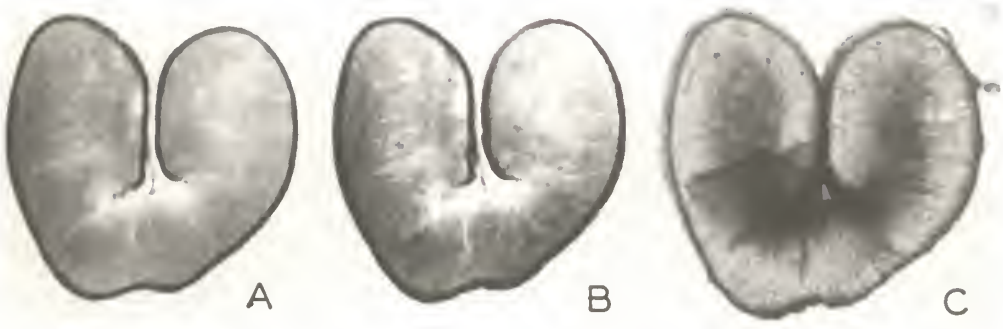
PLATE V



EXPLANATION OF PLATE VI

Shown are positive enlargements of radiographs obtained with fluorescent radiation (A) of wavelength λ_{Ti} at 15 PKV, 20 ma x 30 min, (B) of wavelength λ_{Ca} at 12.5 PKV, 25 ma x 100 min, and (C) a transmitted light photomicrograph. The specimen is a transverse section of Selkirk hard red spring wheat of thickness 360 μ.

PLATE VI



endosperm cells of Omar white wheat are much smaller than those of the other species investigated. To investigate the possibility of calcium concentration within the endosperm cell walls of this variety would require a much thinner section than those used and a very fine grain photographic emulsion.

QUANTITATIVE MEASUREMENTS

The working equation for monochromatic x ray quantitative analysis of calcium derived in appendix I is

$$m_{Ca} = \frac{\left(\frac{\lambda_{Ti}}{\lambda_{Ca}}\right)^{n} \ln\left(\frac{D_{o2} - D_o}{D_2 - D_o}\right) - \ln\left(\frac{D_{o1} - D_o}{D_1 - D_o}\right)}{\left(\frac{\lambda_{Ti}}{\lambda_{Ca}}\right)^{n} \mu_{11} - \mu_{12}}$$

where m_{Ca} is the mass per unit area of calcium, λ_{Ti} and λ_{Ca} are as defined before, D_o is x ray film darkness due to fogging, D_{o1} and D_{o2} are respectively the x ray film darknesses outside the specimen due to incident x ray beams of wavelengths λ_{Ti} and λ_{Ca}, D_1 and D_2 are respectively x ray film darknesses due to x ray beams of wavelengths λ_{Ti} and λ_{Ca} after their passage through the specimen, μ_{11} and μ_{12} are respectively, 880 cm^2/gm and 150 cm^2/gm, the x ray mass absorption coefficients of calcium at λ_{Ti} and λ_{Ca} and $n = 2.8$.

To check the quantitative technique paper specimens were soaked in a solution of $Ca(NO_3)_2.4H_2O$ and distilled water, and the calcium concentration per unit area ($\mu gm/mm^2$) was computed

from massings of the dried paper and an area determination. Two such specimens were prepared, specimen A having 1.26 $\mu gm/mm^2$ of calcium and specimen B having 6.26 $\mu gm/mm^2$ of calcium. A separate radiograph of each artificially doped specimen was obtained with titanium K, λ_{Ti}, and calcium K, λ_{Ca}, radiation. Four such radiograph pairs of specimen A and two of specimen B were obtained. The calcium concentration was computed from darkness measurements taken from these radiographs and an unexposed developed piece of film and was then checked with the massing determination. The results of these computations are presented in Plate VII, table I, where it is seen that calcium concentrations computed from the radiographs are accurate to within $\pm$ 10%.

Accuracy was limited by statistical variation in the film sensitivity, the purity of the x ray beam, and the stability of the Photovolt 520-M photographic densitometer and Model 52 transmission density unit used to obtain these data. An error analysis has indicated that densitometer instability alone causing a variance in blackness measurement of 0.01 results in an error of 0.23 $\mu gm/mm^2$ in the calcium determination, and a variance in blackness measurement of 0.001 yields an error of 0.02 $\mu gm/mm^2$. In the present work the variance in blackness measurements was approximately 0.002 units, corresponding to an average densitometer error in calcium determination of 0.05 $\mu gm/mm^2$.

EXPLANATION OF PLATE VII

Data obtained from the radiographic determination of calcium content of known samples.

TABLE I

RADIOGRAPHIC DETERMINATION OF CALCIUM CONTENT OF KNOWN SAMPLES

Sample	Number*	Calcium content, $\mu gm/mm^2$		Deviation(massing-radiography)	
		by massing	by radiography	$\mu gm/mm^2$	percent
A	A1	1.25	1.31	-.06	-4.8
A	A2	1.25	1.20	.05	4.0
A	A3	1.25	1.28	-.03	-2.4
A	A4	1.25	1.34	-.09	-7.2
B	B1	6.26	6.86	-.60	-9.6
B	B2	6.26	5.86	.40	6.4

* A1 means the pair of radiographs no.1 of paper sample A.

PLATE VII

RESULTS

The calcium concentration as computed using equation (8) and appropriate darkness measurements, in micrograms per mm^2, in small areas of sections from seven different wheat kernels appear in Plate VIII, Table II. Measurements were made in different endosperm regions (3) (outer, cheek, center, and crease) as shown in Plate IX. The choice of the place at which the calcium content was measured in the same region of different kernels was determined by such factors as pitting of the sections, and the distribution of the calcium segregation.

The measurement in the outer region of the Wichita specimen is 8 times as great as the average macroscopic calcium content of 0.05% obtained by other investigators (8). Most other measurements are 2-3 times this macroscopic average but some are in close agreement. The larger values are not surprising, for we have measured specimens having no detectable calcium concentrations.

Circular densitometer apertures of diameters 0.3 mm and 0.8 mm were used to obtain radiographic darkness. The Wichita and Triumph specimens listed in Table II were measured with the smaller aperture. Thus in the Triumph specimen calcium present in volumes of .018 mm^3 was determined. In a volume this size which measured 0.2% Ca there would be 0.05 µgm of calcium, a quantity which is at the lower limit of microbalance capabili-

EXPLANATION OF PLATE VIII

Data obtained from the radiographic determination of calcium content in different regions of thin sections of wheat endosperm from seven different kernels.

TABLE II

CALCIUM CONCENTRATION IN $\mu GM/MM^2$ (AND IN PERCENT)**

Sample	Outer	Cheek	Center	Crease	Thickness
Omar 1	0.9(0.22%)	N.D*(0.00%)	0.8(0.18%)	0.3(0.08%)	317 u
Omar 2	0.4(0.08%)	N.M+(0.00%)	0.2(0.04%)	0.3(0.06%)	351
Selkirk 1	1.1(0.22%)	0.8(0.16%)	0.7(0.14%)	1.7(0.34%)	364
Selkirk 2	0.3(0.06%)	N.M.(0.00%)	0.5(0.10%)	0.8(0.16%)	361
Lee	0.5(0.13%)	0.3(0.07%)	0.5(0.13%)	0.5(0.13%)	288
Wichita	2.5(0.40%)	0.5(0.08%)	0.7(0.11%)	1.4(0.23%)	452
Triumph	0.9(0.26%)	0.7(0.20%)	0.8(0.23%)	1.0(0.29%)	258

* None Detectable. + Not Measured.

** Assigned density of 1.35 gm/cm^3 at 14% moisture.

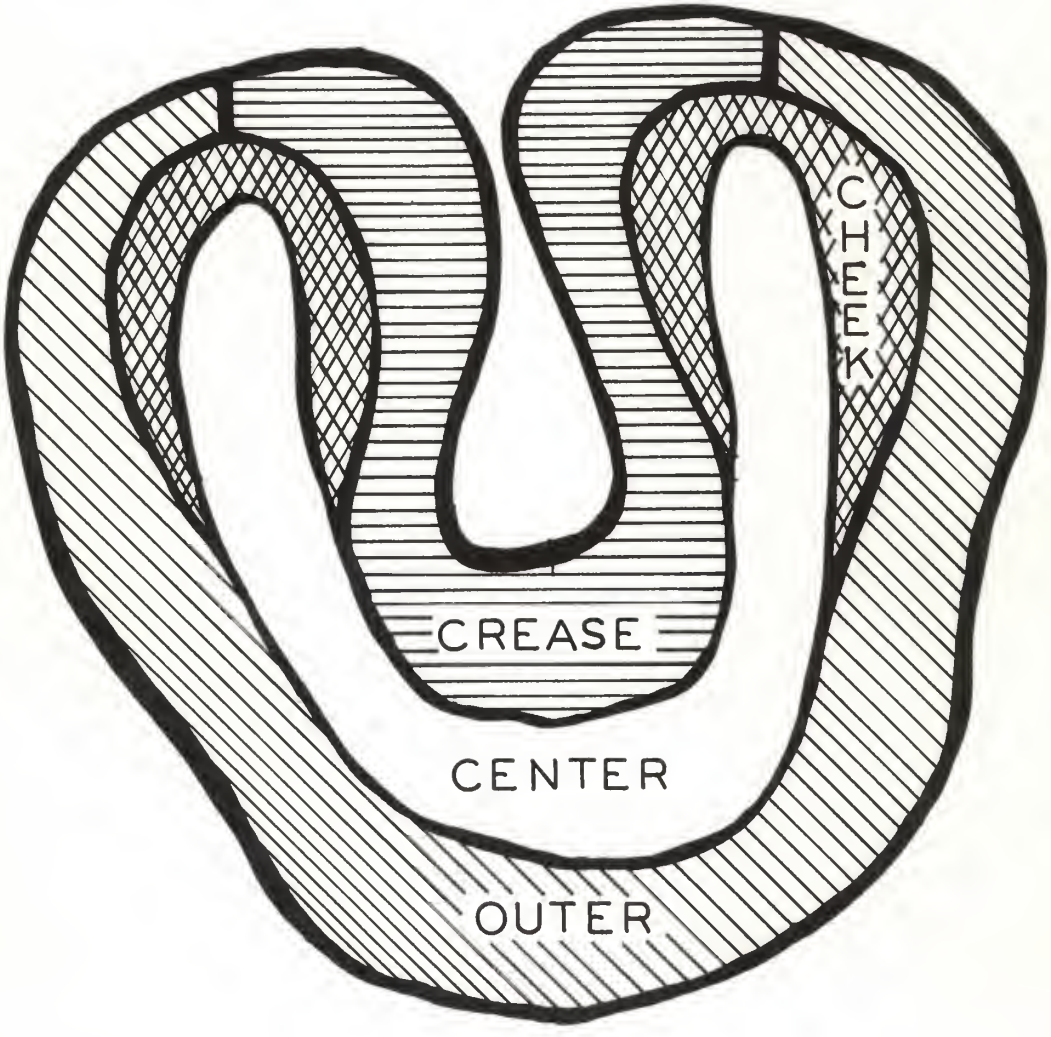
PLATE VIII

EXPLANATION OF PLATE IX

A schematic drawing of a transverse section of a wheat kernel showing the four different endosperm regions.

PLATE IX

ENDOSPERM REGIONS



ties.

SPECIMENS

Kernels which were sectioned and analyzed were chosen from the samples listed in Table III.

TABLE III
SAMPLE ORIGIN AND CROP YEAR

Variety	Species	Origin*	Year
Selkirk	Hard Red Spring	Newell, S.D. Fargo, N.D. Williston, N.D. Crookston, Minn. Boseman, Mont.	1963
Lee	Hard Red Spring	Same As Selkirk	1963
Wichita	Hard Red Winter	Manhattan, Ks. Hutchinson, Ks. Canton, Ks. Hays, Ks. Colby, Ks. Garden City, Ks.	1960
Triumph	Hard Red Winter	Lake Blackwell and Woodward, Okla.	1960
Omar	White	Pullman, Wash.	1963

* Kernels selected were from composite samples from the places indicated.

APPARATUS

The Fluorescent X Ray Attachment

A schematic drawing of the fluorescent x ray attachment constructed for this work is shown in Plate X. The design of the attachment evolved from the necessity to minimize scattering of the primary x ray beam. A is a threaded brass plug which attaches to the Anode shield of a Machlett AEG-50 beryllium window x ray tube; B is a lead lined x ray port which partially collimates the polychromatic primary x ray beam used to irradiate the secondary fluorescent x ray target C; E is a helium inlet to the cylinderical helium chamber D; and F is a 0.00025" mylar diaphragm which closes the lower end of the helium chamber. The mylar diaphragm and I, a polyethylene disk, serve as a cassett which holds the specimen G in contact with the x ray film. The helium gas within D was maintained at a small positive gauge pressure; this prevents the specimen from moving and assures good specimen film contact. The helium path was used to reduce absorption and scattering of the fluorescent x rays.

Secondary fluorescent x ray targets consisted of elements calcium ($z = 20$) through zinc ($z = 30$) with the exception of scandium ($z = 21$). Metals were used in most cases, but when they were not available oxides of the elements were used. The oxides were placed in a paraffin matrix and shaped as shown by C in Plate

EXPLANATION OF PLATE X

An actual size schematic of the fluorescent x ray attachment.

A - Threaded brass plug

B - Primary x ray port

C - Fluorescent x ray target

D - Cylinderical helium chamber

E - Helium inlet

F - Mylar plastic diaphram

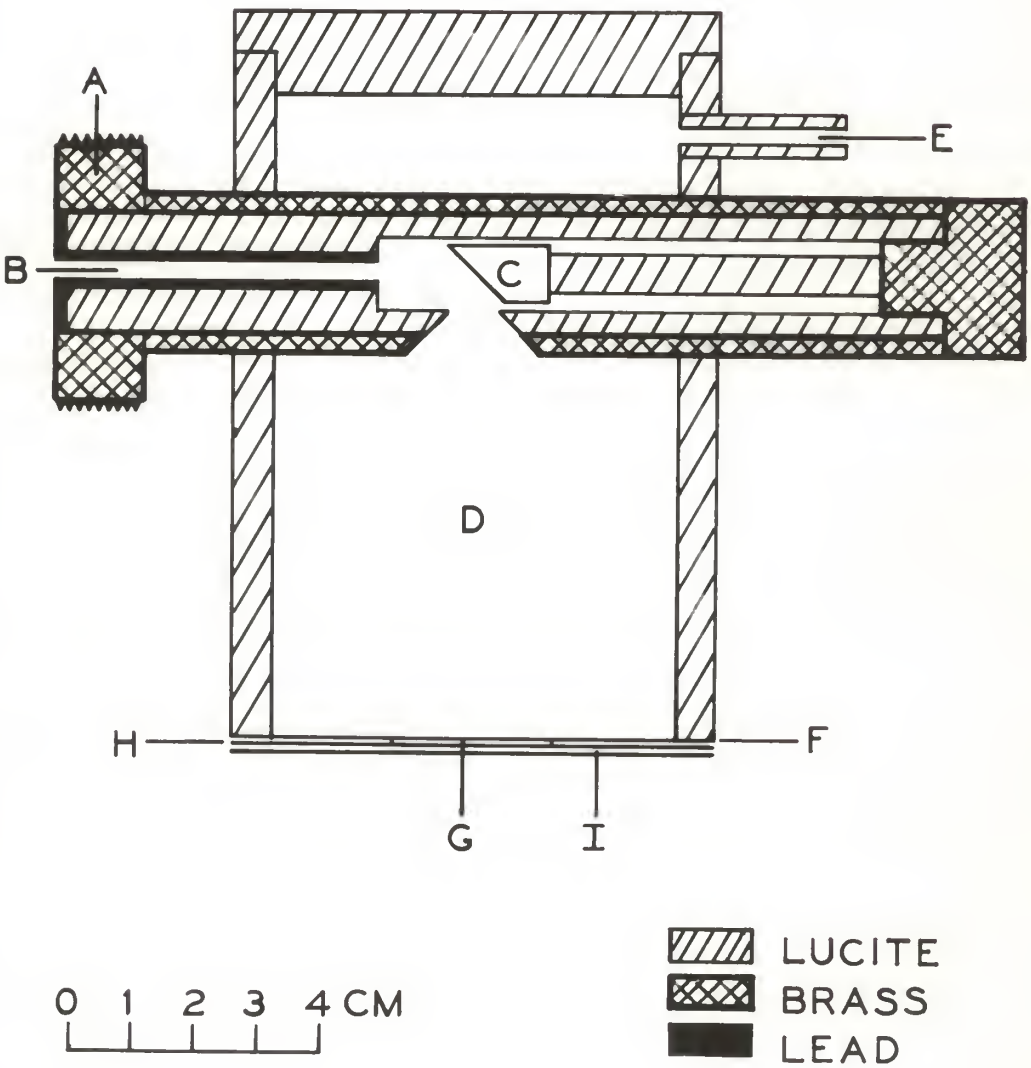
G - Specimen

H - X ray film

I - Polyethylene disk

PLATE X

FLUORESCENT X-RAY ATTACHMENT



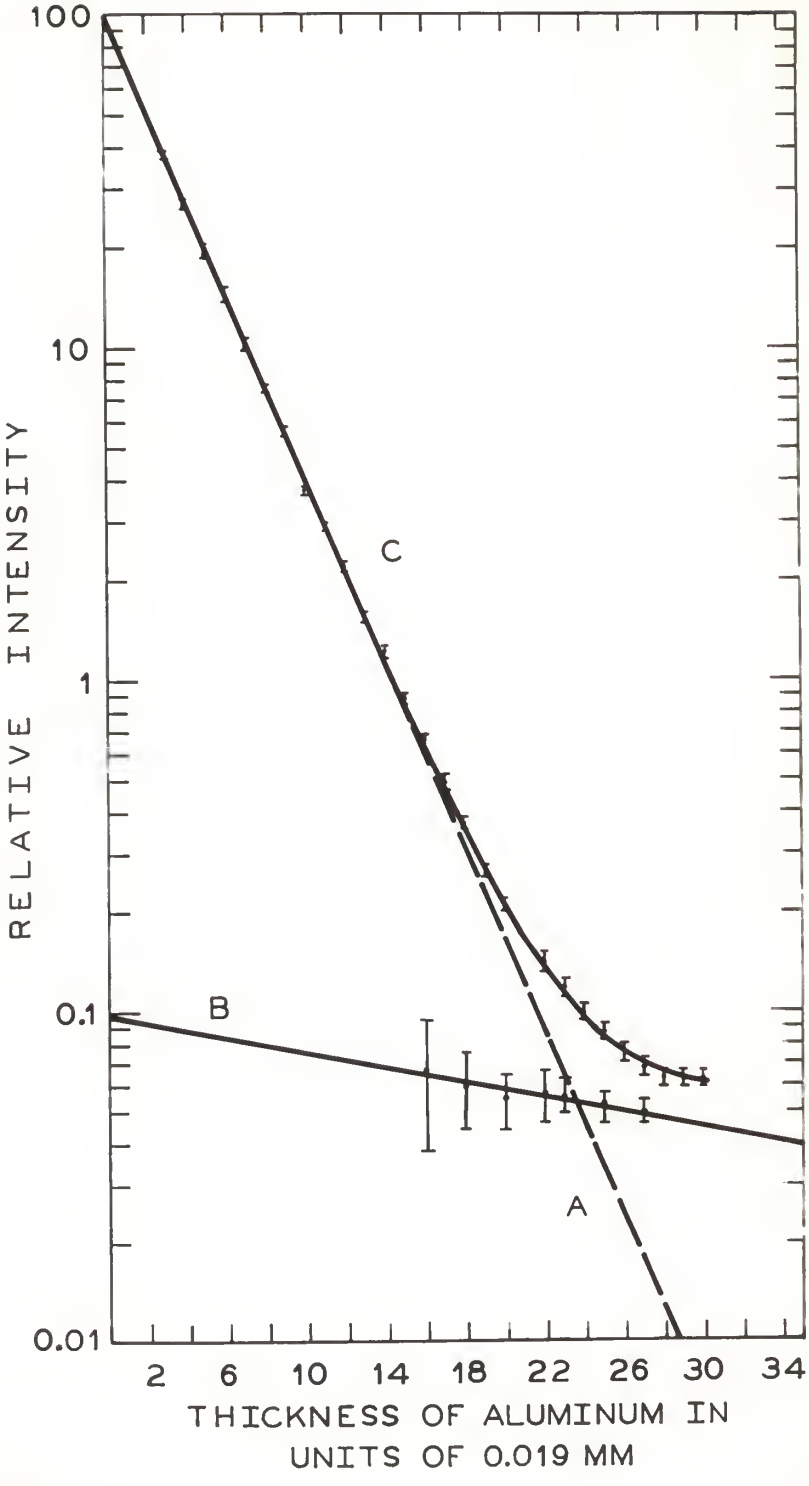
X.

The monochromaticity of the fluorescent beams was checked by plotting aluminum absorption curves for the fluorescent radiation from a Cobalt secondary target, as shown in Plate XI. Here the experimental absorption curve was obtained by use of an aluminum foil step block, and photographic film detection exposed in its linear response range (see Appendix I). The Relative Intensity was plotted against the thickness of aluminum and was decomposed into two straight lines. One line, A, represents the fluorescent radiation and the other line, B, the scattered radiation from the secondary target. The absorption coefficient obtained for the fluorescent component was, 63.5 cm^2 per gm, in reasonable agreement with the tabular value of 73 cm^2 per gm for the absorption of radiation of this wavelength in aluminum. The point at which line B intersects the ordinate, 0.1, is the relative intensity of the radiation scattered from the secondary target. The absorption coefficient represented by this curve was 5 cm^2 per gm, which is consistent with a wavelength of 0.7 Å. This wavelength lies within the continuous spectrum of the primary beam. In this experiment the tungsten target x ray tube was excited to a peak voltage of 20.3 KV corresponding to a maximum intensity wavelength of 0.93 Å. Thus from Figure 3 we find that within the uncertainty of the experiment the beam is 99.9% monochromatic.

EXPLANATION OF PLATE XI

Curve C is a semi-log plot of an experimental aluminum absorption curve for fluorescent Cobalt K radiation. Curve A represents the fluorescent radiation and curve B scattered radiation. Exposures were at 20.3 PKV and 20 ma.

PLATE XI


100
10
1
0.1
0.01
RELATIVE INTENSITY
C
B
A
2
6
10
14
18
22
26
30
34
THICKNESS OF ALUMINUM IN
UNITS OF 0.019 MM

The Densitometer

A photovolt 520-M transmission densitometer was used to determine x ray film density. Density readings are obtained directly with this apparatus, and with properly exposed radiographs, and a mirror scale indicating meter, can be read to an accuracy of 0.002.

This apparatus was turned on and allowed to stablize over a 24 hour period prior to use. Also the photomultiplier tube had to be fatigued at least two hours by exposure to a light intensity slightly greater than the maximum intensity which was to be measured if x ray film density measurements were to be reproducible.

CONCLUSIONS

The information reported shows that fluorescent x ray radiography can be used to map and measure calcium segregations within thin sections of wheat endosperm radiographically by bracketing the K critical absorption edge of calcium with two monochromatic fluorescent x ray beams. The physical basis for this radiographic technique, and the results of some experimental applications are reported. The results of these experiments demonstrate the nonuniformity of calcium distribution within wheat endosperm, and show calcium to be concentrated within the endosperm cell walls.

The quantitative data, for calcium concentration, is accurate to $\pm$ 10%.

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APPENDIX I

Quantitative Measurements Using X Rays

The definitions of the symbols used in the following text are as follows:

λ_i : The wavelength of a monochromatic x ray beam.

I_{oi} : Intensity of an x ray beam of wavelength λ_i before passage through the specimen.

I_i : Intensity of an x ray beam of wavelength λ_i after passage through the specimen.

D_{oi} : Radiographic darkness due to an x ray beam of wavelength λ_i before passage through the specimen.

D_i : Radiographic darkness due to an x ray beam of wavelength λ_i after passage through the specimen.

D_o : Radiograph fogging due to developer, etc.

μ_i : Total x ray mass absorption coefficient of the specimen at x ray wavelength λ_i.

a_j : The fractional mass of the specimen which is comprised of the element of the j^{th} kind.

m : Total mass per unit area of the specimen.

m_j : Mass per unit area of the j^{th} element in the specimen.

k_j : Proportionality constant for the j^{th} element which relates u_{ji} to λ_i^n.

Suppose a biological specimen is comprised of k elements and the mass per unit area of the j = 1 element is to be measured. The total x ray mass absorption coefficient of a polyelemental specimen at an x ray wavelength λ_i is given by

$$\mu_i = a_1 \mu_{1i} + \sum_{j=2}^{k} a_j \mu_{ji} \quad . \tag{1}$$

If λ_1 and λ_2 are chosen such that they bracket the K absorption edge of calcium, but no x ray absorption edges of the elements j = 2,3,... k are bracketed by these wavelengths, then for the j^{th} element $\mu_{ji} = k_j \lambda_i^n$, and therefore

$$\mu_{j2} = \left(\frac{\lambda_1}{\lambda_2}\right)^n \mu_{j1} \tag{2}$$

where n is a number close to 3.

Writing equation (1) for λ_2 and multiplying each term of the resulting equation by $\left(\frac{\lambda_1}{\lambda_2}\right)^n$, and then using (2) it is seen that

$$\left(\frac{\lambda_1}{\lambda_2}\right)^n \mu_2 = \left(\frac{\lambda_1}{\lambda_2}\right)^n a_1 \mu_{12} + \sum_{j=2}^{k} a_j \mu_{j1} \quad . \tag{3}$$

Writing equation (1) for λ_1 and subtracting the resulting equation from (3) the following equation is obtained

$$\left(\frac{\lambda_1}{\lambda_2}\right)^n \mu_2 - \mu_1 = a_1 \left[\left(\frac{\lambda_1}{\lambda_2}\right)^n \mu_{12} - \mu_{11}\right]. \tag{4}$$

Now for a monochromatic x ray beam of wavelength λ_1 passing through a polyelemental specimen

$$\mu_1 = \frac{1}{m} \ln \left(\frac{I_{01}}{I_1} \right) , \tag{5}$$

and for an x ray beam of wavelength λ_2

$$\mu_2 = \frac{1}{m} \ln \left(\frac{I_{02}}{I_2} \right) . \tag{6}$$

Making these substitutions for μ_1 and μ_2 into equation (4) and solving for m_1 ($a_1 \; m = m_1$) yields

$$m_1 = \frac{\left(\frac{\lambda_1}{\lambda_2} \right)^n \ln \left(\frac{I_{02}}{I_2} \right) - \ln \left(\frac{I_{01}}{I_1} \right)}{\left(\frac{\lambda_1}{\lambda_2} \right)^n \mu_{12} - \mu_{11}} . \tag{7}$$

In Plate XII it is shown for film darknesses of 0 - 2.5 that the (x ray beam intensity) x (time) product, the x ray exposure, is a linear function of the radiograph darkness for Kodak type M x ray film. Now since $D - D_0 = KIt$ equation (7) becomes

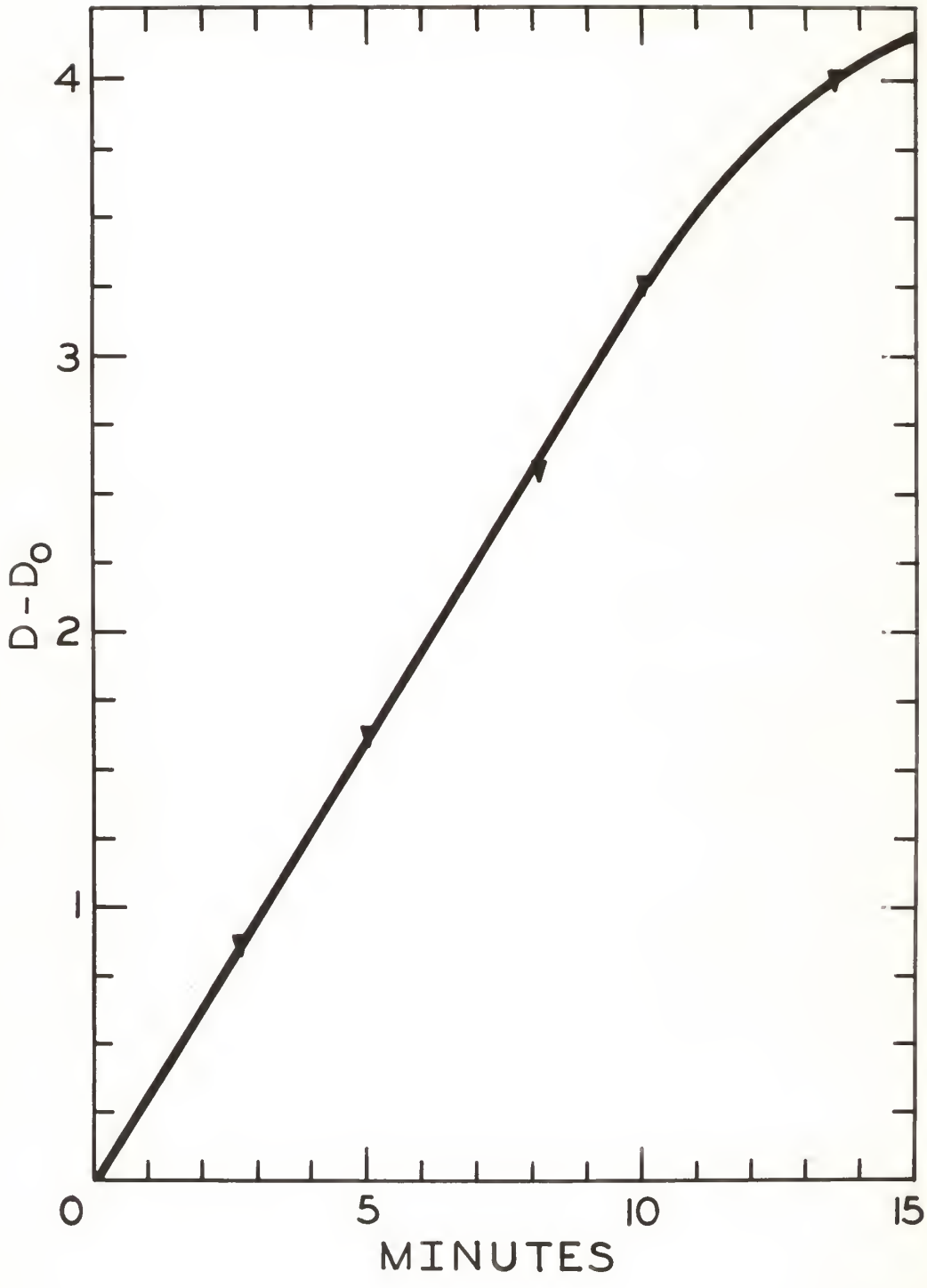
$$m_1 = \frac{\left(\frac{\lambda_1}{\lambda_2} \right)^n \ln \left(\frac{D_{02} - D_0}{D_2 - D_0} \right) - \ln \left(\frac{D_{01} - D_0}{D_1 - D_0} \right)}{\left(\frac{\lambda_1}{\lambda_2} \right)^n \mu_{12} - \mu_{11}} . \tag{8}$$

Equation (8) is the working equation for fluorescent x ray quantitative analysis. The quantities labeled D are measured on the x ray film with a densitometer, and λ_i and μ_{ij} have been determined by others and are tabular, for example, as in the Handbook of Chemistry and Physics.

EXPLANATION OF PLATE XII

This is a plot for Kodak Type M x ray film of radiograph density versus exposure time. The fluorescent x ray beam intensity was held constant. Thus, this graph demonstrates that over a considerable density range $D - D_o$ is directly proportional to the x ray intensity x time product. D_o is the radiograph fogging due to the developer. Iron K radiation was used to make this plot.

PLATE XII



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MONOCHROMATIC X RAY RADIOGRAPHIC ANALYSIS OF CALCIUM IN WHEAT

by

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Monochromatic fluorescent x rays were used to map the positions of calcium segregations within thin sections of wheat endosperm radiographically, and to determine the amount of calcium present in volumes of endosperm as small as 0.018 mm^3. Calcium content in volumes this size was found to be as great as 8 times the chemical macroscopic average of 0.05% obtained by other investigators.

When a material is irradiated with a carefully chosen polychromatic x ray beam from a thin beryllium window x ray tube, monochromatic fluorescent x rays characteristic of this material are produced. Based on this phenomenon a specially designed fluorescent attachment was constructed for the calcium analysis. Monochromaticity of the fluorescent beams obtained with this attachment was demonstrated by plotting an experimental aluminum absorption curve for Cobalt K_α fluorescent radiation. Within the accuracy of the experiment it was shown that 99.9% monochromatic beams were obtainable, and thus were adequate for the calcium analysis.

The wavelength at which the K critical absorption edge of calcium occurs is independent of chemical composition, and in this wavelength region a variation in wavelength changes the x ray absorption coefficient of plant material by only a small amount. This physical phenomenon is exploited for the calcium analysis by bracketing the K edge of calcium with two monochromatic

x ray beams, and noting absorption differences radiographically. These absorption differences appear on a radiograph as visual contrast differences and thus pinpoint calcium segregations. In some specimens, investigated using this technique, concentration of calcium was seen to be within the endosperm cell walls.

The quantitative measurements were made from the radiographs by measuring darknesses with a densitometer, and then placing these measurements into an equation, in the form of a computer program, which gave the calcium concentration per unit area. Results of these computations were tabulated, and show calcium to be nonuniformly distributed within the wheat endosperm.

A check of the quantitative technique was made by radiographically measuring the calcium content of artificially doped paper specimens. The results of this check show the quantitative data to be accurate to $\pm$ 10%.